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TOXICITY OF PHOSPHINE TO 4TH INSTAR LARVAE OF SIX DIFFERENT POPULATIONS OF *Trogodermagranarium* (Everts) (COLEOPTERA:DERMESTIDAE) COLLECTED FROM GODOWNS IN PUNJAB (PAKISTAN)

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Grain pests are causing huge damage to stored grains throughout the world including Pakistan. Among these storage insect pests, *Trogodermagranarium* (Everts) is one of the most threatening pest of stored grains. Present study was conducted to determine the toxicity of Phosphine to 4th instar larvae of six different populations i.e. [(Chishtian (Chi), Haroonabad (Hbd), Lahore (Lhr), Faqeerwali (Fqw), Khaniwal (Khw) and Rawalpindi (Rwp)] of *T. granarium* were collected from various regions of Punjab province. Another major objective was to determine the biochemical and metabolic differences in susceptible and resistant populations of this pest. After collection, they were transferred to lab and used to develop pure lab culture at 30±1°C and 65±5% R.H. of these test populations for further experiments. Mortality was calculated by Lloyd (1969) criterion "insect was judged to be dead when the pressure from a brush failed produces a response". The results were subjected to probit analysis described by Finney (1971). The LC_{50} values were then derived and expressed in ppm of fumigant for 4th instar larvae. Then the mortality data was subjected to logit analysis using POLO-PC (LeOra Software, 1987) to estimate different lethal concentrations up to LC_{90} and confidence limit and regression lines.

Results revealed that a positive co-relationship exist between mortality and concentration of phosphine. The maximum LC_{50} value (7.6ppm) was shown by 4th instar larvae of Hbd population. Fourth instar larvae of Khw were least resistant with LC_{50} at 3.8ppm which indicated that this was the most susceptible population. The LC_{50} value of other population's lye between Hbd and Khw. Fourth Instar larvae of Chi population was the second most resistant population with 7ppm LC_{50}. Doses of phosphine required for 50% mortality in 4th instar larvae of other populations were 6.7ppm (Lhr), 4.7ppm and 5.6ppm (Fqw). On the basis of above LC_{50} results it was concluded that Chi, Hbd and Lhr were resistant populations while Fqw, Khw and Rwp are considered as susceptible populations.

Keywords: Phosphine, Toxicity, *Trogodermagranarium*, 4th Instar, LC_{50} values.

INTRODUCTION

The khapra beetle, *Trogodermagranarium* (Everts), is considered as one of the most significant stored product pests worldwide. In Pakistan *T. granarium* is one of the major pests of stored grains especially wheat. It damage by directly feeding on grains (Azeem *et al*., 1976; Khattak *et al*., 1996; Ram and Singh, 1996). Khapra beetle is also an important pest affecting international trade among uninfested countries, while infested countries suffer major damage through loss of stored grain both in quality and quantity. Young larvae usually attack the embryo point or a weak place in the pericarp (Pasquerault *et al*., 2008) or feed on damaged seed, while older larvae feed on whole grains. Finding khapra beetles in imported commodities will lead an immediate quarantine of the infested goods followed by either rejection or chemical treatment. Pest control chemicals used incorrectly or for prolonged periods may select for pesticide resistance. The continuous and indiscriminate use of pesticides has resulted in resistance development and field control failures in China, India, Japan and Taiwan (Flores *et al*., 2006; Corbel *et al*., 2007; Djouaka *et al*., 2007; Jirakanjanakit *et al* ., 2007; Margaritopoulos *et al*., 2007; Montella, 2007; Oliveira *et al.*, 2007; Stara and Kocourek, 2007).

The cost and residues associated with fumigants is lower than of contact or systemic insecticides. There are many different fumigants e.g., methyl bromide, aluminium phosphide, chloropicrin, magnesium phosphide, sulfuryl fluoride and ethyl formate. However phosphine has proven to be the most widely used.

Fumigants enter the insect's body via respiratory system and depends on fumigant application, temperature and concentration; mortality rate should be 100%. Fumigants have become the most successful method for controlling stored grain pests. Many scientists have studied the application and effectiveness of fumigants to control stored grain pests (Bell and Wilson, 1995; Rajendran and Muralidharan, 2001).

Unfortunately, several cases of phosphine resistance have been reported from Indonesia, UK, India, Philippines, Australia and China (Pike, 1992). A resistance survey carried out by FAO in the early 1970s detected resistance to phosphine in 33 out of 82 countries. (Champ and Dyte, 1976). Since then, many new reports of resistance have emerged in other stored grain pests around the world.

The khapra beetle has developedtolerance to many surface insecticides due to its secretive nature hiding in crevices. It has also developed resistance to phosphie, similar to *S.oryzae, R. dominica. T. castaneum, Cryptolestes spp.* (Srivastava, 1980; Pacheco *et al.,* 1990; Zettler, 1990; Savvidou*et al*., 1994; Zulkifly*et al.,* 1994; Mills and Athie, 2000a, 2000b; Athie*et al*., 2001). Keeping in view the above facts the current study was initiated to evaluate the effect of fumigation against the 4th instar larvae of different populations of *T. granarium.*

MATERIALS AND METHODS

***Rearing and Maintenance of Beetles*:** Fresh cultures of *T. granarium*(Everts) were collected from wheat godowns of Lahore, Khaniwal, Chistian, Rawalpindi, Faqeerwali and Harronabad cities.

Crushed wheat was used as a supporting medium. Wheat was initially fumigated with phosphine to kill the insects if any present. Following fumigation, wheat was spread in fresh air for 4-5 h. The wheat was placed in oven overnight at 60°C, and then shifted into sterilized jars for rearing. The jars were quarter-filled with wheat and 50 beetles were added. The jars were covered with muslin cloth to prevent escape of beetles and entry of other organisms. The beetles were transferred to new jars after 2 days, to maintain the age of larvae for experimental purposes. Wheat containing eggs was replaced in the same jars so that 4th instar larvae were obtained after 32±1, days. Only 4th instar larvae were used for toxicological studies.

The susceptible strain of *T. granarium* was developed in the Biochemistry Laboratory of the University of Punjab, Lahore. The insects were collected from godowns where farmers had never used any kind of pesticides and fumigant to protect them from pests. However, these were reared and bred up to 22 generations at 30±1°C, 65±5% R.H. to get an exact susceptible strain treated as control.

***Toxicants Used*:** The generic name of this chemical isphosphine while hydrogen phosphide and phosphorus trihydride are the common names of phosphine gas. The EPA Chemical Code of this insecticide is 066500. It belongs to Inorganic Phosphine Family.

Phosphine is the only widely used, cost-effective, rapid acting fumigant that does not leave significant residues on the stored product.

***Procedure Adopted*:** The first thing to do for LC_{50} determination was the generation of phosphine gas, which was done according to the technique given in FAO method (Plant protection Bulletin, 1975).

Phosphine was generated from aluminiumphosphide tablets, collected over acidified water. Three glass vials, containing ten healthy larvae of 4th instar of *T. granarium*in each, were placed in the desiccators. Gas was injected into desiccators with micro syringe through a rubber septum fitted on the desiccator lid. The desiccators were kept in the lab at 30±1°C and 65±5% R.H. for 20 h after which observations on mortality were made.

The percentage of killed age was corrected by Abbott's formula (Abbott, 1925). The criterion for mortality was that described by Lloyd (1969). Data was analyzed by the method outlined by Busvine (1971) and described by Finny (1971). Each treatment along with control was repeated thrice.

Mortality data was subjected to logit analysis using POLO-PC (LeOra Software, 1987) to estimate different lethal concentrations up to LC_{90} and confidence limit and regression lines (in ppm Phosphine) for 4th instar larvae of *T. granarium*. Mortality at different concentrations (0-90), used to estimate the concentration-mortality curves.

***Biochemical Analysis*:** About 90 (post treatment) larvae of each instar were homogenized in 0.89% saline with a help of motor driven glass homogenizer under cold conditions. Four replicates of each treatment were used throughout biochemical experimentation. The homogenate was centrifuged at 4200xg for 45 min.

The supernatant thus obtained was used for the estimation of various enzyme activities and other metabolites;

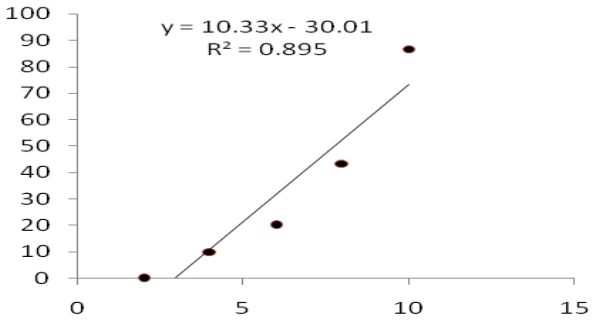
Acid phosphates (AcP: orthophoshoric monoester phosphohydrolase, acid optimum, EC:3.1.3.2) activity according to Andersch and Szcypinski (1947); Alkaline phosphates (AkP; orthophosphoric monoester phosphohydralase alkaline optimum EC: 3.1.3.1) activity as mentioned in Besey*et al*. (1946); lactate dehydrogenase (LDH; L-lactate NAD: oxidoreductase; EC: 1.1.1.27) activity by a method based on Cabaud and Wroblewski (1958); Isocitrate dehydrogenase (ICDH); Threo-Ds-isocitrate: NADP: oxidoreductase, EC: 1.1.1.42) activity by a procedure described by Bell and Baron (1960); Asparatate aminotransferase (ASAT: L-asparatate: 2-oxoglutrate aminotransferase, EC: 2.6.1.1 and alanine aminotransferase E: 2.6.1.2) and Alanine aminotransferase (EC: 2.6.1.2) activities according to Reitmann and Frankel (1957).

RESULTS

In Chi population, the LC_{50} of 4th instar larvae is 7 ppm, while in Fqw population the LC_{50} of its 4th instar larvae was 33.93%. In Hbd population, the LC_{50} for 4th instar larvae was 7.6 ppm. In Khw population, the LC_{50} for 4th instar larvae was 3.8 ppm and In Lhr population, the LC_{50} for 4th instar larvae 25.37% while in Rwp population, the LC_{50} for 4th instar larvae was 4.7 ppm.

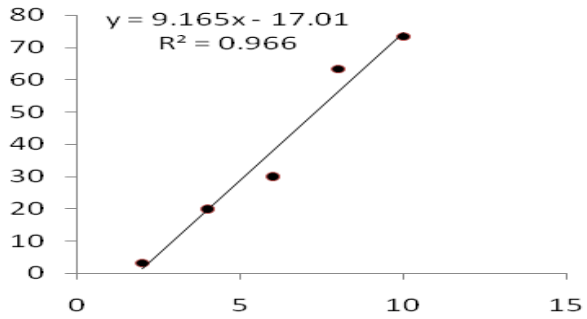
The LC_{50} Values of Chi, Fqw, Hbd, Khw, Lhr, Rwp were showed that Hbd, Lhr and Chi are resistant populations whileFqw, Khw and Rwp are susceptible populations. The most resistant population is Hbd and most susceptible population is Khw population.

Figure 1-6 showed the LC_{50} values of six different strains which helped to differentiate the resistant and susceptible stains of *T. granarium.*



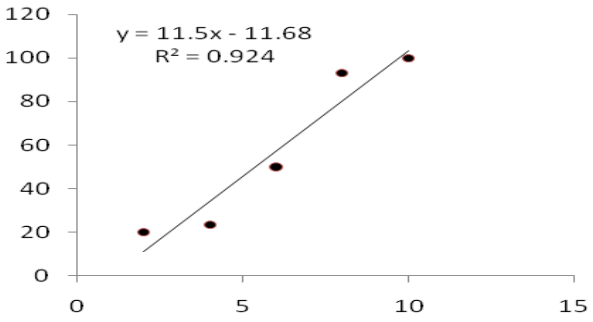
Phosphine treatment (ppm)

Figure 1: LC_{50} of phosphine against 4th instar larvae of *T. granarium*(Haroonabad population)



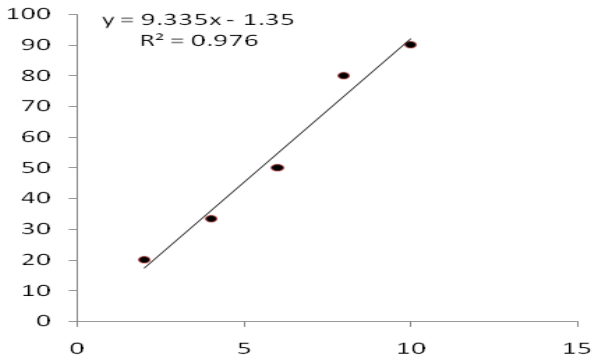
Phosphine treatment (ppm)

Figure 2: LC_{50} of phosphine against 4th instar larvae of *T. granarium*(Chistian Population)



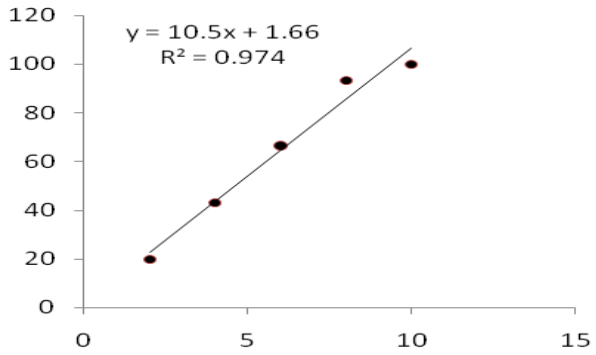
Phosphine treatment (ppm)

Figure 3: LC_{50} of phosphine against 4th instar larvae of *T. granarium*(Lahore Population)



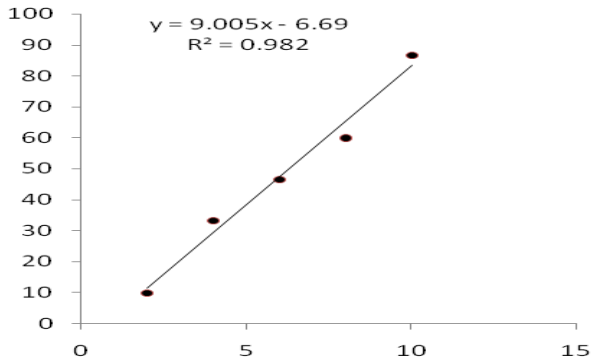
Phosphine treatment (ppm)

Figure 4: LC_{50} of phosphine against 4th instar larvae of *T. granarium*(Rawalpindi Population)



Phosphine treatment (ppm)

Figure 5: LC_{50} of phosphine against 4th instar larvae of *T. granarium*(Khaniwal Population)



Phosphine treatment (ppm)

Figure 6: LC_{50} of phosphine against 4th instar larvae of *T. granarium*(Faqeerwali Population)

***Acid Phosphatase*:** At all above doses, AcP activity decreased gradually in resistant populations (Chishtian (Chi-R1), Haroonabad (Hbd-R2), Lahore (Lhr- R3) which ranges from 0.118±0.002 to 0.128± 0.002 IU/mg. Highly significant decrease of 43% was observed in Hbd population. In

susceptible populations (Faqeerwali (Fqw-S1), Khaniwal (Khw-S2) and Rawalpindi (Rwp-S3)) AcP activity raised at 20ppm except Fqw. Significant increase of 22.76% was observed in Rwp population (Fig. 7).

***Alkaline Phosphatase*:** AkP activity in 4th instar larvae continuously decreased in Lhr, Hbd and Chi populations by 32 to 52%, 20 to 42% and 20 to 36% respectively at 10, 20 and 30 ppm with respect to control ranges from 0.480 ± 0.027 to 0.570 ± 0.011 as shown in Fig. 8. While in all susceptible populations increase was observed at 10, 20 and 30ppm respectively, after 20 hrs exposure of phosphine. A Decrease 0.250± 0.01 IU/mg in Lhr and increase 0.740 ±0.023 IU/mg in Fqw population on 30 ppm showed significant results.

***Amino Transferases*:** ALAT (Fig.10) and ASAT (Fig. 11) activity showed almost same results in resistant and susceptible populations. In all resistant populations (Lhr, Hbd and Chi populations) ALAT activity increased at 30 ppm by 12.40, 8.28 and 13.44% respectively. There was a significant increase in CH population. In susceptible populations (Rwp and Fqw populations) ALAT activity decreased by 9.82% and 2.61% respectively. Khw population showed significant result. ASAT activity decreased in all susceptible populations (Khw, Rwp and Fqw populations) by 5.37, 10.26 and 32.12% at 10 ppm, 8.29, 20.51 and18.13% at 20 ppm doses respectively. ASAT activity rose in all resistant populations (Lhr, Hbd and Chi populations) by 35.32, 3.73 and 13.27% at 10ppm and 45.77, 21.53 and 19.48% at 20ppm respectively. Significant increase was observed in Lhr population and decrease in Rwp population as clear from Fig. 11. At 30ppm ASAT activity showed variations, it increased in Lhr and Chi populations by 48.26% and 29.43% respectively while in all other populations (Khw, Hbd and Rwp populations) ASAT activity decreased significantly by 24, 38 and 37%.

***Dehydrogenases*:** All resistant populations showed decrease activity of LDH (Fig. 12) and ICDH (Fig. 13) while susceptible population indicated the enhanced activity of both enzymes at all doses 10, 20 and 30ppm of phosphine on 4th instar larvae of *T. granarium*. Variations which were observed indicated that decrease was significant in LDH activity at 30ppm in Lhr and Hbd populations by 16.97 and 10.41% as shown in table 1.23. Only Fqw population showed decrease of LDH activity at 30ppm which was 4.58%. In all resistant populations (Chi, Hbd and Lhr populations) ICDH activity decreased at all doses 10ppm, 20ppm and 30ppm of phosphine by 20, 18, 18.5% at 10ppm, 54, 50, 51% at 20ppm, 78, 72 and 73% at 30ppm respectively. Decrease was highly significant at 30ppm (Fig. 13).

Figure 7- 12 showed the effect of phosphine on activities of various enzymes in 4th instar larvae of *T. granarium* at10, 20 and 30ppm doses after the exposure of 20 hours.

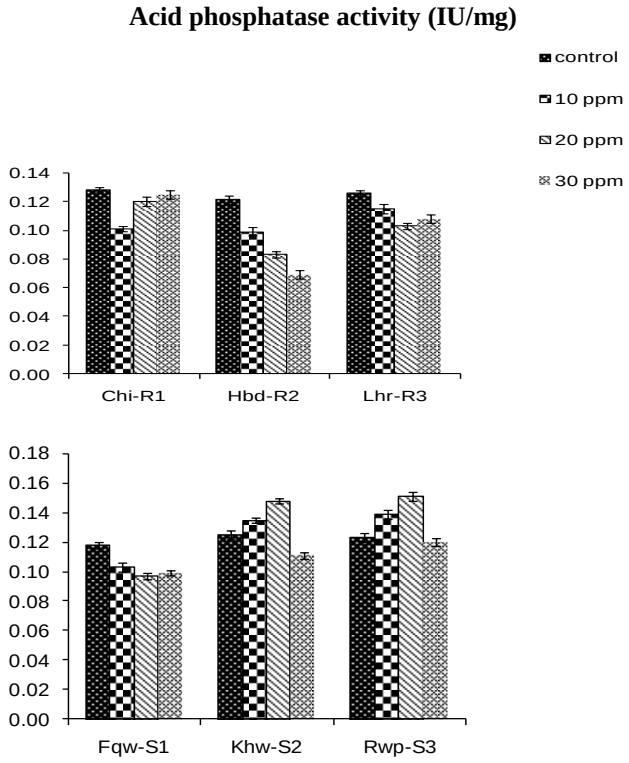


Figure 7: Effect of phosphine (10,20 and 30 ppm) on AcP activity of 4 th instar larvae of *T. granarium*

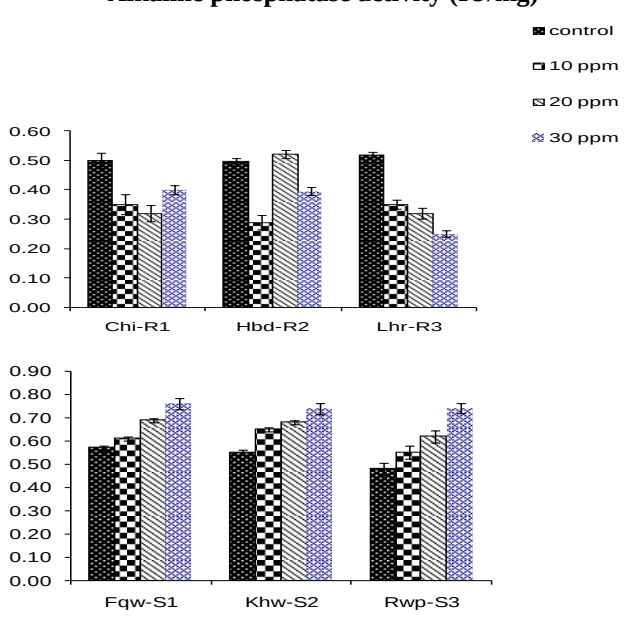


Figure 8: Effect of phosphine (10,20 and 30 ppm) on AkP activity of 4 th instar larvae of *T. granarium*

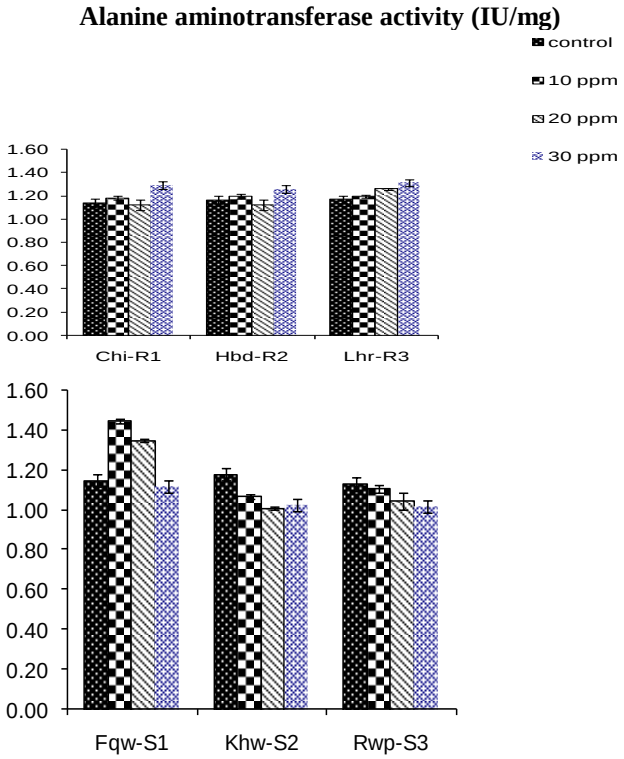


Figure 9: Effect of phosphine (10,20 and 30 ppm) on ALAT activity of 4 th instar larvae of *T. granarium*

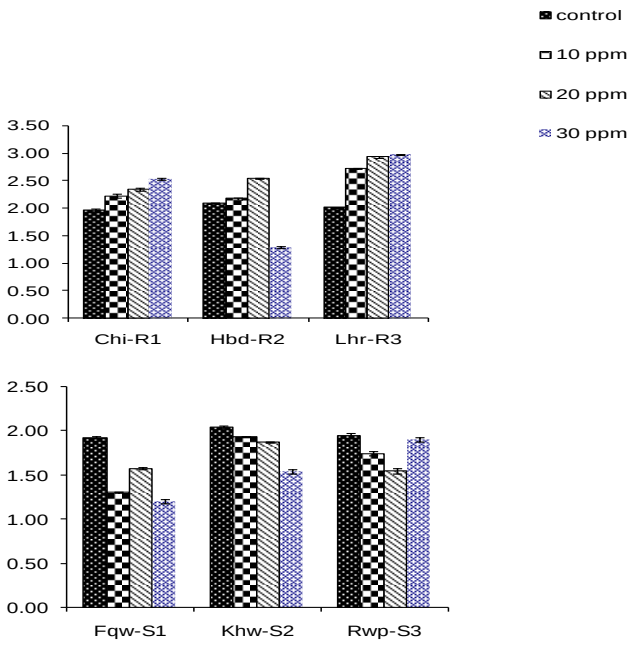


Figure 10: Effect of phosphine (10,20 and 30 ppm) on ASAT activity of 4 th instar larvae of *T.granarium*

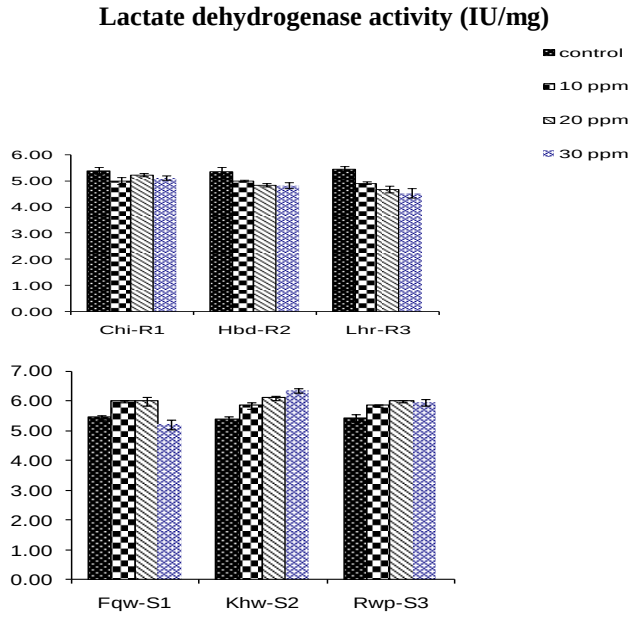


Figure 11: Effect of phosphine (10,20 and 30 ppm) on LDH activity of 4 th instar larvae of *T. granarium*

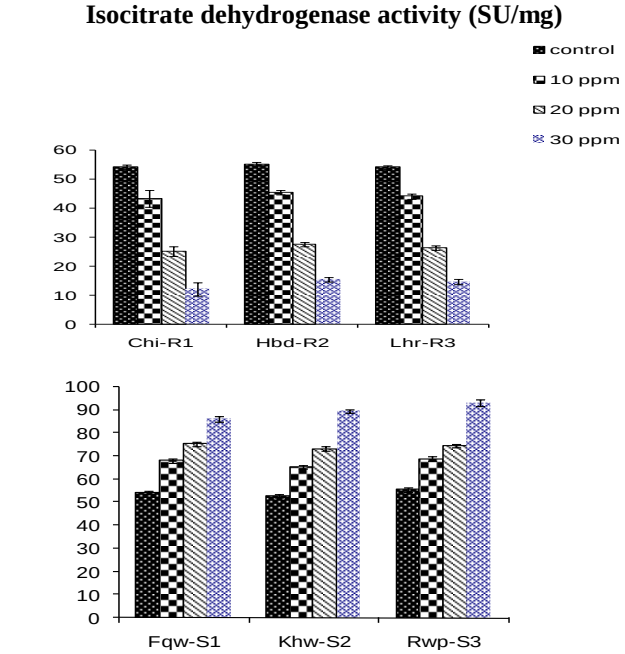


Figure 12: Effect of phosphine (10,20 and 30 ppm) on ICDH activity of 4 th instar larvae of *T. granarium*

DISCUSSION

The results of present study suggested that almost all the enzymes (AcP, AkP, amylase, ALAT, ASAT, LDH, ICDH)

were found to be sensitive in phosphine treated and un-treated 4th instar larvae of *Trogodermagranarium* showing correlation of phosphine with induction/ inhibition of enzymes. Six different populations Chi, Hbd, Lhr, Fqw, Khw and Rwp of *Trogodermagranarium* were collected from different godowns of Punjab have been quantified after the exposure of phosphine for 20 hrs.

This is the first study of its kind on *Trogodermagranarium* (Khapra beetle) in Pakistan. From other laboratories effects of some insecticides have been reported on transaminases of *Culexfatigans* (Srivastava and Verma, 1980), on inhibition of phosphomonoesterases in desert locust (*Schistocercagregaria*) with DDT (Naqvi *et al*., 1970), inactivation of LDH by organochlorines (Meany and Pocker, 1979) and inhibition of trehalase activity in haemolymph of *Phormiareegina* (Friedman, 1961) etc. Shakoori*etal*. (1989) reported effects of different insecticides, mixture of insecticides on the enzyme, metabolites and macromolecules of 6th instar larvae of *T. castaneum*.

***Aliesterases (Acp, Akp)*:** AkP activity showed significant increase at 30ppm in all larvae of all populations for increased energy requirement which results to overcome the sudden toxic stress (Shakoori and Saleem, 1999; Dow and Davis, 2001; Yi and Adams, 2001; Cabero *et al*., 2004) While AcP activity was inhibited at 20 ppm in 4th instar larvae of all populations. In resistant populations aliesterases decreased in larval stages in all populations (Fig. 7-17). Similar results are described by Ashfaq *et al*., (2004) in adult beetles of *T. castaneum*after the treatment of cypermethrin insecticide at sub lethal dose. In contrast to this study, Venkateswara. (2006) indicated that, due to organophosphorus insecticides, AcP and AkP was inhibited in *Oreochromismossambicus* while exposure duration was 3, 7, 15 and 30 days. Reduced activity of phosphatases also reported by Shakoori *et al*. (1993, 1999).

***Aminotransferases*:** Induced activities of aminotransferases (ASAT and ALAT) may probably be due to induction process at molecular level to route amino acids into Kreb's cycle for production of energy or for gluconeogenesis in resistant populations of 4th instar larvae. These enzymes normally indicative of aminoacid catabolism and promote breakdown of aminoacids by transfer of amino groups to keto acids. Ghousia. (2003) and Venkateswara. (2006) describe the similar response of these two aminotransferases in *Clariasbetrachus*and in *Oreochromismossambicus* respectively in response to carbofuran and organophosphorus insecticides.

***Dehydrogenases*:** Significant decrease in LDH activity was found in current studies at 30 ppm of phosphine in resistant populations of 4th instar larvae of *T. granarium* in Chi, Hbd and Lhr populations (Fig. 12) this decrease indicate lower respiration rate through inhibition of lactate to pyruvate inter conversion. Similar results were described by Byrne *et al*., (2003) and Venkateswara (2006). LDH activity also decreased in *T. castaneum* due to diffusion of phosphine in haemolymph of insects (Khan, 1989). In susceptible populations (Fqw, Khw and Rwp) LDH activity enhanced to support the respiration Like susceptible larval LDH activity increased in 4th larvae of *Tribolium castaneum* in response to cyhalothrin, Karate (the pyrethroids) and other insecticides (Saleem and Shakoori,1985 & 1986; Shakoori *et al*., 1988; Shakoori and Saleem,1989).

***Conclusion*:** Reduced levels of ICDH in 4th instar larvae of resistant populations (Hbd, Lhr and Chi), suggested that citric acid cycle was probably deactivated or slowed down, which provided less energy to the insect at both the doses of 20 ppm and 30 ppm of phosphine, ICDH activity was enhanced in susceptible populations of larvae (Fqw, Khw and Rwp). Which revealed that citric acid cycle more active in this stage. The findings of this study was supported by Shakoori *et al*. (1987, 1989, 1999) working on *T.castaneum* with organophosphates.

Data produced during these experiments can be used as indicator of phosphine toxicity and contribute to understanding the mechanism of toxicity in *T. granarium*.

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